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(54) Title: METHODS OF ALLEVIATING NEUROPATHIC PAIN USING PROSAPOSIN-DERIVED PEPTIDES

(57) Abstract

The invention provides a method of alleviating or preventing neuropathic pain in a subject by administering an effective amount of an active fragment of prosaposin to the subject. The invention also provides prosaposin-derived fragments and the use of these fragments for stimulating neurite outgrowth, inhibiting neural cell death, promoting myelination and inhibiting demyelination. In addition, there is provided a method of inhibiting sensory or motor neuropathy by contacting neuronal cells with a composition comprising an effective amount of an active fragment of prosaposin.

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CERTIFICATE OF CORRECTION

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INVENTOR(S) : Fu-Kuen Lin

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 38, line 52, "amount an" should be --amount of an--.

Signed and Sealed this

Twenty-seventh Day of January, 1998

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METHODS OF ALLEVIATING NEUROPATHIC PAIN USING PROSAPOSIN-DERIVED PEPTIDES

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

This invention relates generally to the field of pain therapy and more specifically to the use of prosaposin-derived peptides for the treatment of neuropathic pain.

BACKGROUND INFORMATION

Neuropathic pain results from injury to a nerve. In contrast to the immediate pain caused by tissue injury, neuropathic pain can develop days or months after a traumatic injury. Furthermore, while pain caused by tissue injury is usually limited in duration to the period of tissue repair, neuropathic pain frequently is long-lasting or chronic. Moreover, neuropathic pain can occur spontaneously or as a result of stimulation that normally is not painful.

The clinical causes of neuropathic pain are widespread and include both trauma and disease. For example, traumatic nerve compression or crush and traumatic injury to the brain or spinal cord are common causes of neuropathic pain. Furthermore, most traumatic nerve injuries also cause the formation of neuromas, in which pain occurs as a result of aberrant nerve regeneration. In addition, cancer-related neuropathic pain is caused when tumor growth painfully compresses adjacent nerves, brain or spinal cord. Neuropathic pain

also is associated with diseases such as diabetes or alcoholism.

Unfortunately, neuropathic pain frequently is resistant to available drug therapies. In addition, current therapies have serious side-effects including, for example, cognitive changes, sedation, nausea and, in the case of narcotic drugs, addiction. Many patients suffering from neuropathic pain are elderly or have other medical conditions that particularly limit their tolerance of the side-effects associated with available drug therapy. The inadequacy of current therapy in relieving neuropathic pain without producing intolerable side-effects frequently is manifest in the depression and suicidal tendency of chronic pain sufferers.

Methods of alleviating neuropathic pain would improve the quality of life for many people suffering from pain due to trauma or disease. However, there currently are no effective drugs that relieve neuropathic pain without undesirable side-effects such as sedation and addiction. Thus, there is a need for methods of alleviating neuropathic pain without producing undesirable side-effects. The present invention satisfies this need and provides related advantages as well.

SUMMARY OF THE INVENTION

The present invention provides a method of alleviating neuropathic pain in a subject by administering an effective amount of an active fragment of prosaposin to the subject. For example, the invention provides a method of alleviating neuropathic pain resulting from a disorder of peripheral nerve, dorsal root ganglia, spinal cord, brainstem, thalamus or cortex in a subject by administering an effective amount of an active fragment of prosaposin having the amino acid sequence Cys-Glu-Phe-Leu-Val-Lys-Glu-Val-Thr-Lys-Leu-Ile-Asp-Asn-Asn-Lys-Thr-Glu-Lys-Glu-Ile-Leu (SEQ ID NO: 1) or Thr-D-Ala-Leu-Ile-Asp-Asn-Asn-Ala-Thr-Glu-Glu-Ile-Leu-Tyr (SEQ ID NO: 2). In addition, the invention provides a method of preventing neuropathic pain in a subject by administering an effective amount of an active fragment of prosaposin to the subject. The present invention also provides prosaposin-derived peptides and the use of these peptides for stimulating neurite outgrowth, inhibiting neural cell death, promoting myelination and inhibiting neural demyelination. In addition, there is provided a method of inhibiting sensory or motor neuropathy by contacting neuronal cells with a composition comprising an effective inhibiting amount of an active fragment of prosaposin.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the threshold of tactile allodynia before (time 0) and at various times after bolus injection of prosaposin-derived 22-mer peptide (SEQ ID NO: 1) in Chung model rats.

Figure 2 shows the threshold of tactile allodynia before (time 0) and at various times after bolus injection of prosaposin-derived 14-mer peptide (SEQ ID NO: 2) in Chung model rats.

Figure 3 shows the sum flinches in response to 0.5% formalin after intraperitoneal administration of prosaposin-derived 14-mer peptide (SEQ ID NO: 2) or saline in diabetic rats.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a method of alleviating neuropathic pain in a subject by administering an effective amount of an active fragment of prosaposin to the subject. As disclosed herein, the method of the invention can alleviate neuropathic pain in a subject within 30 minutes of administration. Such a method is useful for alleviating neuropathic pain resulting from a disorder of peripheral nerve, dorsal root ganglia, spinal cord, brainstem, thalamus or cortex.

A peptide useful in the invention is derived from prosaposin, which is a 517 amino acid protein originally identified as the precursor of four sphingolipid activator proteins (Kishimoto et al., J. Lipid Res., 33:1255-1267 (1992)). Four adjacent tandem domains in prosaposin are proteolytically processed in lysosomes to generate saposins A, B, C, and D, which activate hydrolysis of glycospingolipids by lysosomal hydrolases (O'Brien and Kishimoto, FASEB J., 5:301-308 (1991)).

The unprocessed form of prosaposin is found in high concentrations in human and rat brain, where it is localized within neuronal surface membranes. During embryonic development, prosaposin mRNA is abundant in brain and dorsal root ganglia. Furthermore, prosaposin binds with high affinity to gangliosides, which stimulate neurite outgrowth, and promotes transfer of gangliosides from micelles to membranes.

The neurotrophic activity of prosaposin is consistent with its localization in neuronal cell populations (O'Brien et al., Proc. Natl. Acad. Sci., USA 91:9593-9596 (1994); Sano et al., Biochem. Biophys. Res. Commun., 204:994-1000 (1994)). Prosaposin stimulates motor neurite outgrowth *in vitro* and *in vivo* and increases choline acetyltransferase activity, which is a marker of neuronal differentiation. In addition, prosaposin prevents cell death in neuroblastoma cells (O'Brien et al., *supra*, 1994; O'Brien et al., FASEB J. 9: 681-685 (1995)).

The neurotrophic activity of prosaposin is localized to saposin C, a domain of 80 amino acids. A 22-mer peptide corresponding to amino acids 8 to 29 of the saposin C domain (SEQ ID NO: 1) stimulates neurite outgrowth and choline acetyltransferase activity and prevents cell death in neuroblastoma cells (O'Brien et al., *supra*, 1995).

Prosaposin or the prosaposin-derived 22-mer peptide (SEQ ID NO: 1), for example, can modulate motor

neuron function by promoting neurite outgrowth. Prior to the present invention, however, it was not known whether prosaposin or a peptide fragment of prosaposin could affect sensory neuron function. Moreover, the neurotrophic activity of prosaposin or a prosaposin-derived peptide in stimulating motor neurite outgrowth is evident only after a period of 24 to 48 hours (see, for example, O'Brien et al., *supra*, 1994). Neurotrophic activity of prosaposin or a prosaposin-derived peptide has not been demonstrated to occur in a shorter period of time.

In contrast, the present invention provides a method of alleviating neuropathic pain, which involves both sensory and motor neuron components. Furthermore, the method of the invention is effective in alleviating neuropathic pain in a matter of minutes rather than the hours or days previously demonstrated to be required for the neurotrophic activity of prosaposin or a prosaposin-derived peptide.

The effectiveness of the method of the invention in alleviating neuropathic pain was demonstrated using the well-recognized Chung rat model of peripheral neuropathy. In the Chung rat model, spinal nerve partial ligation of left spinal nerves L-5 and L-6 produces a long-lasting hypersensitivity to light pressure on the affected left foot. The hypersensitivity is similar to the pain experienced by humans with the neuropathic condition of causalgia as described in Kim and Chung, Pain 50:355-363 (1992).

Prior to administration of an active fragment of prosaposin, Chung model rats had a threshold of 3.0 to 4.0 g before the affected foot was withdrawn in response to pressure (Von Frey hairs) (see Figure 1). After administration of an active fragment of prosaposin (prosaposin-derived 22-mer; SEQ ID NO: 1), neuropathic pain was alleviated, as evidenced by a greater tolerance to pressure before the affected foot was withdrawn. The effect of the active fragment of prosaposin occurred within 15 minutes and was sustained for 3 hours following administration as shown in Figure 1. This rapid relief of neuropathic pain is in stark contrast to the delayed neurotrophic effects previously reported for prosaposin and peptides derived from prosaposin.

An active fragment of prosaposin such as the prosaposin-derived peptide SEQ ID NO: 2 also alleviated pain in a rat model of painful diabetic neuropathy. As described in Example III, peptide SEQ ID NO: 2 reduced allodynia in rats with short-term insulin-deficient diabetes induced by the selective β cell toxin, streptozotocin. Thus, an active fragment of prosaposin or a prosaposin-derived peptide of the invention can be used to alleviate a variety of types of neuropathic pain including mechanical pain, as exemplified by the Chung rat model, and metabolic pain, as exemplified by the use of these peptides in reducing pain in diabetic rats.

As used herein, the term "neuropathic pain" means pain resulting from injury to a nerve. Neuropathic pain is distinguished from nociceptive pain, which is the

pain caused by acute tissue injury involving small cutaneous nerves or small nerves in muscle or connective tissue. Pain involving a nociceptive mechanism usually is limited in duration to the period of tissue repair and generally is alleviated by available analgesic agents or opioids as described in Myers, Regional Anesthesia 20:173-184 (1995).

Neuropathic pain typically is long-lasting or chronic and often develops days or months following an initial acute tissue injury. Neuropathic pain can involve persistent, spontaneous pain as well as allodynia, which is a painful response to a stimulus that normally is not painful. Neuropathic pain also can be characterized by hyperalgesia, in which there is an accentuated response to a painful stimulus that usually is trivial, such as a pin prick. Unlike nociceptive pain, neuropathic pain generally is resistant to opioid therapy (Myers, *supra*, 1995).

The method of the invention is useful in alleviating neuropathic pain resulting from a disorder of peripheral nerve, dorsal root ganglia, spinal cord, brainstem, thalamus or cortex. As used herein, the term "disorder" means any trauma, injury, disease or condition resulting in neuropathic pain.

The method of the invention is useful in alleviating neuropathic pain regardless of the etiology of the pain. For example, a method of the invention can be used to alleviate neuropathic pain resulting from a

peripheral nerve disorder such as neuroma; nerve compression; nerve crush, nerve stretch or incomplete nerve transsection; mononeuropathy or polyneuropathy. A method of the invention also can be used to alleviate neuropathic pain resulting from a disorder such as dorsal root ganglion compression; inflammation of the spinal cord; contusion, tumor or hemisection of the spinal cord; tumors of the brainstem, thalamus or cortex; or trauma to the brainstem, thalamus or cortex (see, for example, Table 1).

The method of the invention can be useful, for example, to alleviate neuropathic pain resulting from a neuroma, which can develop readily after traumatic injury to nerve, especially when a whole nerve is severely crushed or transsected. In a neuroma, the neurite outgrowth that normally regenerates a peripheral nerve is aberrant or misguided due, for example, to a physical obstruction such as scar tissue. Thus, a regenerating nerve fiber is entangled in an environment in which mechanical and physical factors precipitate abnormal electrophysiologic activity and pain (Myers, *supra*, 1995). An amputation neuroma, for example, can cause phantom pain or can cause pain triggered by the use of a limb prosthesis. As disclosed herein, such neuropathic pain can be alleviated by administration of an active fragment of prosaposin according to a method of the invention.

Nerve compression also results in neuropathic pain that can be treated using the method of the

Table 1
Nerve Neuroma (amputation, nerve transsection) Nerve compression (entrapment neuropathies, tumors) Nerve crush, stretch or incomplete transsection (trauma) Mononeuropathy Diabetes mellitus Irradiation Ischemia Vasculitis Polyneuropathy Post-polio syndrome Diabetes mellitus Alcohol Amyloid Toxic HIV Hypothyroidism Uremia Vitamin deficiencies Chemotherapy (vincristine, cisplatinum, paclitaxel) ddC (zalcitabine) Fabry's disease
Dorsal root ganglion Compression (disk, tumor, scar tissue) Root avulsion Inflammation (postherpetic neuralgia)
Spinal cord Contusion Tumor Hemisection
Brainstem, thalamus, cortex Infarction, tumors, trauma

invention. Nerve compression can be abrupt, as in the case of traumatic nerve crush, or can be prolonged and moderate, secondary to tumor growth or scar formation in the proximity of a major nerve bundle. Compression neuropathy can occur as a result of changes in blood flow

to a nerve, causing severe ischemia and consequent nerve injury (Myers, *supra*, 1995).

Administration of an active fragment of prosaposin according to the method of the invention also can alleviate neuropathic pain resulting from a mononeuropathy or polyneuropathy. As used herein, a neuropathy is a functional disturbance or pathological change in the peripheral nervous system and is characterized clinically by sensory or motor neuron abnormalities. The term mononeuropathy indicates that a single peripheral nerve is affected, while the term polyneuropathy indicates that several peripheral nerves are affected.

The etiology of a neuropathy can be known or unknown (see, for example, Myers, *supra*, 1995; Galer, Neurology 45(suppl 9):S17-S25 (1995); Stevens and Lowe, Pathology, Times Mirror International Publishers Limited, London (1995)). Known etiologies include complications of a disease or toxic state; for example, diabetes is the most common metabolic disorder causing neuropathy. The method of the invention alleviates the neuropathic pain of a mononeuropathy resulting, for example, from diabetes, irradiation, ischemia or vasculitis. The method of the invention also alleviates the neuropathic pain of a polyneuropathy resulting, for example, from post-polio syndrome, diabetes, alcohol, amyloid, toxins, HIV, hypothyroidism, uremia, vitamin deficiencies, chemotherapy, ddC or Fabry's disease (see Table 1). The method of the invention particularly is useful in

alleviating post-polio myalgia. The method of the invention also can alleviate neuropathic pain of unknown etiology.

As disclosed herein, an active fragment of prosaposin also can be useful in alleviating neuropathic pain or in stimulating neurite outgrowth, inhibiting neural cell death, promoting myelination or inhibiting demyelination or in inhibiting sensory neuropathy. The term "active fragment of prosaposin," as used herein, means a peptide that has an amino acid sequence corresponding to an amino acid sequence of prosaposin and that has activity in alleviating neuropathic pain or in stimulating neurite outgrowth, inhibiting neural cell death, promoting myelination or inhibiting demyelination or in inhibiting sensory or motor neuropathy.

As used herein, alleviating neuropathic pain means reducing the severity of neuropathic pain. In a human subject, an active fragment of prosaposin reduces the severity of neuropathic pain such that the subject's suffering is diminished and quality of life is improved. An active fragment of prosaposin also can alleviate neuropathic pain in any one of a number of well-established animal models of neuropathic pain as described further below (also see Bennett, Muscle & Nerve 16:1040-1048 (1993)). As used herein, the term "active fragment of prosaposin" is synonymous with "prosaposin-derived peptide".

The active fragment of prosaposin preferably contains the amino acid sequence Leu-Ile-Asp-Asn-Asn-Lys-Thr-Glu-Lys-Glu-Ile-Leu (SEQ ID NO: 3), which corresponds to amino acids 18 to 29 of saposin C. More preferably, an active fragment of prosaposin has the amino acid sequence Cys-Glu-Phe-Leu-Val-Lys-Glu-Val-Thr-Lys-Leu-Ile-Asp-Asn-Asn-Lys-Thr-Glu-Lys-Glu-Ile-Leu (SEQ ID NO: 1), which corresponds to amino acids 8 to 29 of saposin C, or the amino acid sequence Thr-D-Ala-Leu-Ile-Asp-Asn-Asn-Ala-Thr-Glu-Glu-Ile-Leu-Tyr (SEQ ID NO: 2), which corresponds to amino acids 16 to 29 of saposin C but which has been modified by a D-alanine for lysine substitution at position 2; an alanine for lysine substitution at position 8; a deletion of lysine at position 11 and the addition of a C-terminal tyrosine residue (see Table 2). Such modifications can be useful for increasing peptide stability or uptake across the blood-brain barrier as described below. As used herein, D-alanine can be represented by D-Ala or X.

An active fragment of prosaposin can have about 12 amino acids to about 80 amino acids, which is the full-length of saposin C. Preferably, an active fragment of prosaposin has about 12 amino acids to about 40 amino acids and, more preferably, about 14 amino acids to about 22 amino acids.

Table 2		
PEPTIDE	**SEQUENCE**	**SEQ ID NO:**
Prosaposin-derived 22-mer	CEFLVKEVTKLIDNNKTEKEIL	1
Prosaposin-derived 14-mer	TXLIDNNATEEILY	2
Prosaposin-derived 12-mer	LIDNNKTEKEIL	3
where X = D-alanine		

For use in alleviating neuropathic pain in a human subject, an active fragment of human prosaposin, such as SEQ ID NO: 1 or SEQ ID NO: 2, is preferred. However, an active fragment derived from another mammalian prosaposin also is useful in alleviating neuropathic pain according to the method of the invention. Thus, for example, an active fragment of mouse prosaposin, rat prosaposin, guinea pig prosaposin or bovine prosaposin such as SEQ ID NOS: 4 through 7 also can be useful in alleviating neuropathic pain in a subject.

The amino acid sequence of an active fragment of human prosaposin (SEQ ID NO: 1), which corresponds to amino acids 8 to 29 of saposin C, is well conserved among other species, as shown in Table 3. In particular, adjacent asparagine (N) residues are conserved among human, mouse, rat, guinea pig and bovine prosaposins. In addition, a leucine (L) residue is conserved 3 to 4 residues toward the N-terminus of the two asparagine residues and one or more charged residues (aspartic acid (D), lysine (K), glutamic acid (E) or arginine (R)) are conserved 2 to 8 residues toward the C-terminus of the

two asparagine residues. Each of these well-conserved residues is underlined in Table 3.

Table 3

SPECIES	SEQUENCE	SEQ ID NO.
Human	CEFLVKEVTKLIDNNKTEKEIL	1
Mouse	CQFVMNKFSELIVNNATEELLY	4
Rat	CQLVNRKLSELIINNATEELL-	5
Guinea Pig	CEYVVKKVMLLIDNNRTEEKII	6
Bovine	CEFVVKEVAKLIDNNRTEEEIL	7

The well-conserved adjacent asparagine residues, leucine residue and charged residues described above can be important for the activity of an active fragment of prosaposin in alleviating neuropathic pain or in stimulating neurite outgrowth, inhibiting neural cell death, promoting myelination or inhibiting demyelination or in inhibiting or motor neuropathy. For example, the prosaposin-derived 22-mer (SEQ ID NO: 1) or the prosaposin-derived 14-mer (SEQ ID NO: 2) is an active fragment of prosaposin, which reduces the painful allodynia seen in the Chung rat model of peripheral neuropathy, as disclosed in Example I (see Figures 1 and 2). In contrast, a mutant 22-mer (SEQ ID NO: 8), which differs from SEQ ID NO: 1 in having an aspartic acid residue (D) in place of the first conserved asparagine (see Table 4), lacks activity in alleviating neuropathic pain as assayed using Chung rats (see Example I).

The activity of a peptide in alleviating neuropathic pain also can correlate with neurotrophic activity. For example, the prosaposin-derived 22-mer (SEQ ID NO: 1) and the prosaposin-derived 14-mer (SEQ ID NO: 2) alleviate neuropathic pain and have neurotrophic

Table 4

PEPTIDE	SEQUENCE	SEQ ID NO:
Prosaposin-derived 22-mer	CEFLVKEVTKLIDNNKTEKEIL	1
Mutant 22-mer	CEFLVKEVTKLIDDNKTEKEIL	8
Prosaposin-derived 14-mer	TXLIDNNATEEILY	2
Mutant 14-mer M-1	TKLIDNDKTEKEIL	9
Mutant 14-mer M-2	TKSIDNNKTEKEIL	10
where X = D-alanine		

activity. In addition, the mutant 22-mer (SEQ ID NO: 8) is inactive in alleviating neuropathic pain as described above and lacks neurotrophic activity, further indicating that activity in alleviating neuropathic pain can correlate with neurotrophic activity. The mutant 14-mer peptide M-1 (SEQ ID NO: 9), which has a substitution of the second conserved asparagine residue, lacks neurotrophic activity, indicating that peptide SEQ ID NO: 9 also is inactive in alleviating neuropathic pain. The mutant 14-mer peptide M-2 (SEQ ID NO: 10), which has a substitution of the conserved leucine residue, lacks neurotrophic activity, indicating that peptide SEQ ID NO: 10 is inactive in alleviating neuropathic pain. In contrast, the prosaposin-derived 12-mer peptide (SEQ ID

NO: 3), which has the conserved adjacent asparagines, leucine and charged residues described above, is active as a neurotrophic factor. Thus, the prosaposin-derived 12-mer peptide (SEQ ID NO: 3) also can alleviate neuropathic pain according to the method of the invention.

Prosaposin-derived peptides, and neurotrophic analogs thereof, possess significant therapeutic applications in promoting functional recovery after toxic, traumatic, ischemic, degenerative or inherited lesions to the peripheral or central nervous system. In addition, these peptides can promote myelination or inhibit demyelination, thereby counteracting the effects of demyelinating diseases. Furthermore, such peptides stimulate the outgrowth of neurons and inhibit programmed cell death in neuronal tissues. The active neurotrophic and myelinotrophic peptides of the invention have between about 12 or 14 and about 50 amino acids and preferably include the non-naturally occurring prosaposin sequence shown in SEQ ID NO: 2. For example, the active neurotrophic and myelinotrophic peptides of the invention have between 14 and about 50 amino acids and include the non-naturally occurring prosaposin sequence shown in SEQ ID NO: 2.

In another embodiment of the present invention, there is provided a method of stimulating neurite outgrowth, inhibiting neural cell death, promoting myelination or inhibiting demyelination in differentiated or undifferentiated neuronal cells by administering to

the neuronal cells an effective amount of a neurite outgrowth or myelin-facilitating peptide having between about 12 and about 50 amino acids and preferably including the peptide shown in SEQ ID NO: 2. In the methods of the invention for stimulating neurite outgrowth, inhibiting neural cell death, promoting myelination or inhibiting demyelination, an effective amount of a peptide having, for example, between 14 and about 50 amino acids and including the peptide shown in SEQ ID NO: 2 can be used.

The ability of any such peptide to stimulate neurite outgrowth, inhibit neural cell death, promote myelination or inhibit demyelination readily can be determined by one skilled in the art using the procedures described in Examples IV to VII. Methods for assaying the abilities of these peptides to promote myelination and to inhibit demyelination are set forth in Examples VI and VII below.

The present invention also provides a method of inhibiting sensory neuropathy by contacting neuronal cells with a composition comprising an effective inhibiting amount of an active fragment of prosaposin. The invention provides, for example, a method of inhibiting sensory neuropathy by contacting neuronal cells with a composition comprising an effective inhibiting amount of a peptide having the sequence shown as SEQ ID NO: 1 or SEQ ID NO: 2.

As described herein in Example X, a prosaposin-derived peptide can be useful in inhibiting sensory neuropathy. In a mouse model in which sensory neuropathy is induced by taxol administration, a loss of thermal sensation is normally seen. However, in taxol-treated mice given 100 μg/kg of peptide SEQ ID NO: 1, the loss of thermal sensation was inhibited. These results indicate that prosaposin-derived peptides can be a neurotrophic factor for both sensory and motor neurons.

A peptide useful in the methods of the invention also can be, for example, SEQ ID NOS: 11 through 19 (see Table 5). For example, sequence alignment of the prosaposin-derived 22-mer peptide SEQ ID NO: 1 with cytokines and growth factors indicates sequence similarity to a number of human (h) cytokines including hCNTF, hIL-6, hIL-2, hIL-3, hIL1-γ, erythropoietin (hEPO), human leukocyte inhibitory factor (hLIF), the hIL-1 β chain and oncostatin-M (hONC-M). SEQ ID NOS: 11 through 19, like the active fragment of prosaposin SEQ ID NO: 1, contain two asparagine residues that are adjacent or separated by one amino acid. In addition, the cytokine-derived peptide sequences can contain a leucine (L) or isoleucine (I) residue three to four residues toward the N-terminus of the two asparagine residues and one or more charged residues (aspartic acid (D), lysine (K), glutamic acid (E), or arginine (R)) two to eight residues toward the C-terminus of the two asparagine residues, as is seen in the active fragment of

prosaposin (22-mer; SEQ ID NO: 1). Each of these residues is underlined in Table 5.

Models of cytokine-receptor binding (Sprang and Bazan, Curr. Opin. Struct. Biol., 3:816 (1993)) have highlighted the evolutionary conservation of a four-helical bundle structure common to many cytokines. Each of the cytokine or growth-factor sequences related to the prosaposin-derived sequence SEQ ID NO: 1 is located between helices A and B (AB loop) or within helix C of the cytokine.

Table 5

CYTOKINE	SEQUENCE	LOCATION	SEQ ID NO:
Prosaposin	CEFLVKEVTKLIDNNKTEKEIL	----	1
hCNTF	YVKHQGLNKNINLDSVDGVP	AB loop	11
hIL-6	EALAENNLNLPKMAG	AB loop	12
hIL-2	LQMILNGINNYKNPKLT	AB loop	13
hIL-3	ILMENNLRRPNL	AB loop	14
hIL1-γ	FYLRNNQLVAGTL	AB loop	15
hEPO	AEHCSLNENITVPDTKV	AB loop	16
hLIF	YTAQGEPFPNNVEKLCAP	AB loop	17
hIL-1β	FNKIEINNKLEFESA	Helix C	18
hONC-M	RPNIGLRNNIYCMAQLL	Helix C	19

The structurally related cytokine and growth factor-derived peptides SEQ ID NOS: 11 through 19 also can be useful in methods of alleviating neuropathic pain. Peptides SEQ ID NOS: 11 through 19 can be assayed for activity in alleviating neuropathic pain using, for

example, the Chung rat model described in Example I; a model of diabetic neuropathy as described in Example III assays described by Wall et al., Pain 7:103-113 (1979); Bennett and Xie, Pain 33:87-107 (1988); Lekan et al., Soc. Neurosci. Abstr. 18:287 (1992) or Palacek et al., Soc. Neurosci. Abstr. 18:287 (1992); or other assays for neuropathic pain.

The cytokine and growth factor-derived peptides SEQ ID NOS: 11 through 19 also can be useful in methods of stimulating neurite outgrowth, inhibiting neural cell death, promoting myelination or inhibiting demyelination or in methods of inhibiting sensory or motor neuropathy. A peptide having between about 14 and about 50 amino acids and including the active neurotrophic region contained within one of sequences SEQ ID NOS: 11 through 19 can be assayed for the ability to stimulate neurite outgrowth as described in Example IV; or assayed for the ability to inhibit neural cell death as described in Example V; or for the ability to promote myelination as described in Example VI; or for the ability to inhibit demyelination as described in Example VII; or for the ability to inhibit sensory neuropathy as described in Example X.

An active fragment of prosaposin or a peptide useful in alleviating neuropathic pain can be identified by screening a large collection, or library, of random peptides or peptides of interest using, for example, one of a number of animal models of neuropathic pain. Such peptides of interest can be, for example, the cytokine

and growth factor-derived peptides SEQ ID NOS: 11 through 19, which have amino acid sequences related to an active fragment of prosaposin (SEQ ID NO: 1). Peptides of interest also can be, for example, a population of peptides related in amino acid sequence to SEQ ID NO: 1 by having the conserved asparagine residues, leucine/isoleucine residue and one or more charged residues at the positions corresponding to the positions in which these residues are found in SEQ ID NO: 1 but also having one or more amino acids that differ from the amino acids of SEQ ID NO: 1.

Peptide libraries include, for example, tagged chemical libraries comprising peptides and peptidomimetic molecules. Peptide libraries also comprise those generated by phage display technology. Phage display technology includes the expression of peptide molecules on the surface of phage as well as other methodologies by which a protein ligand is or can be associated with the nucleic acid which encodes it. Methods for the production of phage display libraries, including vectors and methods of diversifying the population of peptides which are expressed, are well known in the art (see, for example, Smith and Scott, Methods Enzymol. 217:228-257 (1993); Scott and Smith, Science 249:386-390 (1990); and Huse, WO 91/07141 and WO 91/07149). These or other well known methods can be used to produce a phage display library, from which the displayed peptides can be cleaved and assayed for activity in alleviating neuropathic pain or other neurotrophic or myelinotrophic activity as described herein. If desired, a population of peptides

can be assayed for activity, and an active population can be subdivided and the assay repeated in order to isolate an active peptide from the population. Other methods for producing peptides useful in the invention include, for example, rational design and mutagenesis based on the amino acid sequences of active fragments of prosaposin such as SEQ ID NO: 1 and SEQ ID NO: 2, for example.

As disclosed herein, an active fragment of prosaposin or a peptide useful in alleviating neuropathic pain can be identified by its activity in alleviating neuropathic pain in any of a number of well-established animal models of neuropathic pain (Bennett, *supra*, 1993). For example, an active fragment of prosaposin can be identified using an experimental model of peripheral neuropathy produced by segmental spinal nerve ligation in the rat. The Chung rat model duplicates the symptoms of human patients with causalgia, or burning pain due to injury of a peripheral nerve (Kim and Chung, *supra*, 1992). The surgical procedure of Kim and Chung produces a long-lasting hyperalgesia to noxious heat and mechanical allodynia of the affected foot. As described in Example I, rats with spinal nerve ligation according to the procedure developed by Chung and Kim are useful for identifying an active fragment of prosaposin for use in alleviating neuropathic pain.

An active fragment of prosaposin or a peptide useful in alleviating neuropathic pain also can be identified by its activity in alleviating neuropathic pain in a rat model of painful diabetic neuropathy.

Hyperalgesia to thermal, mechanical and chemical noxious stimuli also has been reported in diabetic rats with short-term insulin-deficient diabetes induced by selective β cell toxins such as streptozotocin (Calcutt et al., Pain 68:293-299 (1996). Such a rat model is representative of the pain evidenced in diabetic humans, who may exhibit a variety of aberrant sensations including spontaneous pain, pain evoked by light touch and hyperalgesia. Rats treated with streptozotocin or another selective β cell toxin can be treated with a fragment or peptide of interest; subsequently, the response to a noxious stimulus such as 0.5% formalin is measured. A reduced response can be used to identify an active fragment of prosaposin or a peptide useful in alleviating neuropathic pain.

An active fragment of prosaposin or a peptide useful in alleviating neuropathic pain also can be identified using the neuroma model of Wall et al. This well-recognized model of neuropathic pain reproduces the human symptoms seen following amputation or nerve transection in an intact limb (Wall et al., *supra*, 1979). As discussed above, a neuroma forms readily after nerve transection due to the frustrated growth of neurite sprouts.

A model of chronic constriction injury also can be used to identify an active fragment of prosaposin or a peptide useful in alleviating neuropathic pain. The chronic constriction injury model of Bennett and Xie, *supra*, 1988, is a rat model of peripheral neuropathy that

produces pain disorders like those seen in man. In the Bennett model, nerve injury is created by loosely tying constrictive ligatures around the rat sciatic nerve, causing degeneration of nerve distal to the constriction. Allodynia and hyperalgesia are produced by the constriction injury in addition to spontaneous pain.

Primate models of neuropathic pain also are useful for identifying an active fragment of prosaposin or a peptide useful in alleviating neuropathic pain (see, for example, Lekan et al., *supra*, 1992; Palacek et al., *supra*, 1992).

As used herein, the term "peptide," as used in reference to an active fragment of prosaposin, a prosaposin-derived peptide or a peptide useful in the methods of the invention, means a compound containing naturally occurring amino acids, non-naturally occurring amino acids or chemically modified amino acids, provided that the compound retains activity in alleviating neuropathic pain or other neurotrophic or myelinotrophic activity as described herein. A prosaposin-derived peptide also can be a peptide mimetic, which is a non-amino acid chemical structure that mimics the structure of a prosaposin-derived peptide and retains activity. Such a mimetic generally is characterized as exhibiting similar physical characteristics such as size, charge or hydrophobicity in the same spatial arrangement found in the prosaposin-derived peptide counterpart. A specific example of a peptide mimetic is a compound in which the amide bond between one or more of the amino

acids is replaced, for example, by a carbon-carbon bond or other bond well known in the art (see, for example, Sawyer, Peptide Based Drug Design, ACS, Washington (1995)).

As used herein, the term "amino acid" refers to one of the twenty naturally occurring amino acids, including, unless stated otherwise, L-amino acids and D-amino acids. The term amino acid also refers to compounds such as chemically modified amino acids including amino acid analogs, naturally occurring amino acids that are not usually incorporated into proteins such as norleucine, and chemically synthesized compounds having properties known in the art to be characteristic of an amino acid, provided that the compound can be substituted within a peptide such that it retains its biological activity. For example, glutamine can be an amino acid analog of asparagine, provided that it can be substituted within an active fragment of prosaposin that retains its activity in alleviating neuropathic pain or other neurotrophic or myelinotrophic activity as described herein. Other examples of amino acids and amino acids analogs are listed in Gross and Meienhofer, The Peptides: Analysis, Synthesis, Biology, Academic Press, Inc., New York (1983). An amino acid also can be an amino acid mimetic, which is a structure that exhibits substantially the same spatial arrangement of functional groups as an amino acid but does not necessarily have both the α-amino and α-carboxyl groups characteristic of an amino acid.

An active fragment of prosaposin or a peptide useful in the invention can be isolated or synthesized using methods well known in the art. Such methods include recombinant DNA methods and chemical synthesis methods for production of a peptide. Recombinant methods of producing a peptide through expression of a nucleic acid sequence encoding the peptide in a suitable host cell are well known in the art and are described, for example, in Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed, Vols 1 to 3, Cold Spring Harbor Laboratory Press, New York (1989).

An active fragment of prosaposin or a peptide useful in the invention also can be produced by chemical synthesis, for example, by the solid phase peptide synthesis method of Merrifield et al., J. Am. Chem. Soc. 85:2149 (1964). Standard solution methods well known in the art also can be used to synthesize a peptide useful in the invention (see, for example, Bodanszky, Principles of Peptide Synthesis, Springer-Verlag, Berlin (1984) and Bodanszky, Peptide Chemistry, Springer-Verlag, Berlin (1993)). A newly synthesized peptide can be purified, for example, by high performance liquid chromatography (HPLC), and can be characterized using, for example, mass spectrometry or amino acid sequence analysis.

It is understood that limited modifications can be made to an active fragment of prosaposin without destroying its biological function. Thus, a modification of an active fragment of prosaposin that does not destroy its ability to alleviate neuropathic pain is within the

definition of an active fragment of prosaposin. A modification can include, for example, an addition, deletion, or substitution of amino acid residues; a substitution of a compound that mimics amino acid structure or function; and addition of chemical moieties such as amino or acetyl groups. The activity of a modified peptide in alleviating neuropathic pain can be assayed using an animal model of neuropathic pain, such as those described above or the assay exemplified in Example I.

A particularly useful modification of an active fragment of prosaposin is one that confers, for example, increased stability. For example, incorporation of one or more D-amino acids or substitution or deletion of lysine can increase the stability of an active fragment of prosaposin by protecting against peptide degradation. For example, as disclosed herein, the prosaposin-derived 14-mer SEQ ID NO: 2 has an amino acid sequence derived from amino acids 16 to 29 of saposin C but which has been modified by substitution or deletion of each of the three naturally occurring lysines and the addition of a C-terminal tyrosine residue. In particular, the prosaposin-derived 14-mer SEQ ID NO: 2 has a D-alanine for lysine substitution at position 2; an alanine for lysine substitution at position 8 and a deletion of lysine at position 11. The D-alanine substitution at position 2 confers increased stability by protecting the peptide from endoprotease degradation, as is well known in the art (see, for example, page 247 of Partridge, Peptide Drug Delivery to the Brain, Raven Press, New York

(1991)). The substitution or deletion of a lysine residue confers increased resistance to trypsin-like proteases, as is well known in the art (Partridge, *supra*, 1991). These substitutions increase stability and, thus, bioavailability of peptide SEQ ID NO: 2, but do not affect activity in alleviating neuropathic pain.

A useful modification also can be one that promotes peptide passage across the blood-brain barrier, such as a modification that increases lipophilicity or decreases hydrogen bonding. For example, a tyrosine residue added to the C-terminus of the prosaposin-derived peptide (SEQ ID NO: 2) increases hydrophobicity and permeability to the blood-brain barrier (see, for example, Banks et al., Peptides 13:1289-1294 (1992) and Pardridge, *supra*, 1991). A chimeric peptide-pharmaceutical that has increased biological stability or increased permeability to the blood-brain barrier, for example, also can be useful in the method of the invention.

One skilled in the art can readily assay the ability of an active fragment of prosaposin to cross the blood-brain barrier *in vivo*, for example, as disclosed in Example II. In addition, an active fragment of prosaposin can be tested for its ability to cross the blood-brain barrier using an *in vitro* model of the blood-brain barrier based on a brain microvessel endothelial cell culture system, for example as described in Bowman et al., Ann. Neurol. 14:396-402 (1983) or Takahura et al., Adv. Pharmacol. 22:137-165 (1992).

As used herein, the term "effective amount" means the amount of an active fragment of prosaposin useful for alleviating neuropathic pain or for preventing neuropathic pain. An effective amount to be administered systemically on a daily basis depends on the body weight of the subject. Preferably, an effective amount to be administered systemically on a daily basis is about 0.1 μg/kg to about 1000 μg/kg. More preferably, an effective amount to be administered systemically on a daily basis is about 10 μg/kg to about 100 μg/kg. An effective amount of a peptide for alleviating or preventing pain can be determined empirically using methods well known to those in the art, including, for example, the assay described in Example I or those disclosed above, including assays in primates (Lekan et al., *supra*, 1992, and Palacek et al., *supra*, 1992).

A typical minimum amount of the peptides of the invention for neurotrophic or myelinotrophic activity in cell growth medium is at least about 5 ng/ml. This amount or more of a peptide of the invention can be used for *in vitro* use. Typically, concentrations in the range of 0.1 μg/ml to about 10 μg/ml of a peptide of the invention can be used. An effective amount for treatment of a particular tissue can be determined as set forth in Examples IV and VI.

Neural cells can be treated *in vitro* or *ex vivo* by directly administering a peptide of the invention to the cells. This can be done, for example, by culturing

the cells in growth medium suitable for a particular cell type, followed by addition of peptide to the medium. When the neural cells to be treated are *in vivo*, typically in a vertebrate, preferably a mammal, a peptide of the invention can be administered by one of several techniques as described below.

As used herein, the term "subject" means a vertebrate, preferably a mammal and, in particular, a human.

The present invention provides methods of alleviating pain, stimulating neurite outgrowth, inhibiting neural cell death, promoting myelination and inhibiting demyelination and methods of inhibiting sensory or motor neuropathy by administering an effective amount of an active fragment of prosaposin intravenously, intramuscularly, intradermally, subcutaneously, intracranially, intracerebrospinally, topically, orally transdermally, transmucosally or intranasally. A pharmaceutically acceptable carrier of well known type can be administered with an active fragment of prosaposin. Such carriers include, for example, phosphate buffered saline (PBS).

Preferably, an effective amount of an active fragment of prosaposin is injected directly into the bloodstream of the subject. For example, intravenous injection of an active fragment of prosaposin can be used to administer the active fragment to the peripheral or central nervous system, since an iodinated

prosaposin-derived 18-mer Tyr-Lys-Glu-Val-Thr-Lys-Leu-Ile-Asp-Asn-Asn-Lys-Thr-Glu-Lys-Glu-Ile-Leu (SEQ ID NO: 20), consisting of amino acids 12 to 29 of prosaposin-derived 22-mer SEQ ID NO: 1 with a substitution of tyrosine for valine at amino acid 12 (MW = 2000) crossed the blood-brain barrier and entered the central nervous system as described in Example II. The uptake by the brain was approximately 0.03%, which is in the midrange of values for peptides of that approximate size that will cross the blood-brain barrier (Banks et al., *supra*, 1992).

Oral administration often can be desirable, provided the active fragment of prosaposin is modified so as to be stable to gastrointestinal degradation and readily absorbable. The substitution, for example, of one or more D-amino acids can confer increased stability to a prosaposin-derived peptide useful in the invention.

Direct intracranial injection or injection into the cerebrospinal fluid also can be used to introduce an effective amount of an active fragment of prosaposin into the central nervous system of a subject. In addition, an active fragment of prosaposin can be administered to peripheral neural tissue by direct injection or local topical application or by systemic administration. Various conventional modes of administration also are contemplated, including intravenous, intramuscular, intradermal, subcutaneous, intracranial, epidural, topical, oral, transdermal, transmucosal and intranasal administration.

An active fragment of prosaposin also can be administered in a sustained release form. The sustained release of an active fragment of prosaposin has the advantage of alleviating neuropathic pain over an extended period of time without the need for repeated administrations of the active fragment.

Sustained release can be achieved, for example, with a sustained release material such as a wafer, an immunobead, a micropump or other material that provides for controlled slow release of the active fragment of prosaposin. Such controlled release materials are well known in the art and available from commercial sources (Alza Corp., Palo Alto CA; Depotech, La Jolla CA; see, also, Pardoll, Ann. Rev. Immunol. 13:399-415 (1995). In addition, a bioerodible or biodegradable material that can be formulated with an active fragment of prosaposin, such as polylactic acid, polygalactic acid, regenerated collagen, multilamellar liposomes or other conventional depot formulations, can be implanted to slowly release the active fragment of prosaposin. The use of infusion pumps, matrix entrapment systems, and transdermal delivery devices also are contemplated in the present invention.

An active fragment of prosaposin also can be advantageously enclosed in micelles or liposomes. Liposome encapsulation technology is well known. Liposomes can be targeted to a specific tissue, such as neural tissue, through the use of receptors, ligands or antibodies capable of binding the targeted tissue. The

preparation of these formulations is well known in the art (see, for example, Pardridge, *supra*, 1991, and Radin and Metz, Meth. Enzymol. 98:613-618 (1983)).

A peptide composition of the invention can be packaged and administered in unit dosage form, such as an injectable composition or local preparation in a dosage amount equivalent to the daily dosage administered to a patient, and if desired can be prepared in a controlled release formulation. Unit dosage form can be, for example, a septum sealed vial containing a daily dose of the active composition of the invention in PBS or in lyophilized form. For treatment of neural diseases, an appropriate daily systemic dosages of a peptide of the invention is based on the body weight of the vertebrate and is in the range of from about 10 to about 100 μg/kg, although dosages from about 0.1 to about 1,000 μg/kg are also contemplated. Thus, for the typical 70 kg human, a systemic dosage can be between about 7 and about 70,000 μg daily and preferably between about 700 and about 7,000 μg daily. A daily dosage of locally administered material will be about an order of magnitude less than the systemic dosage. Oral administration is also contemplated.

The invention also provides a method of alleviating neuropathic pain in a subject by transplanting into the subject a cell genetically modified to express and secrete an active fragment of prosaposin. Transplantation can provide a continuous source of an active fragment of prosaposin and, thus,

sustained alleviation of neuropathic pain. For a subject suffering from prolonged or chronic neuropathic pain, such a method has the advantage of obviating or reducing the need for repeated administration of an active fragment of prosaposin.

Using methods well known in the art, a cell readily can be transfected with an expression vector containing a nucleic acid encoding an active fragment of prosaposin (Chang, Somatic Gene Therapy, CRC Press, Boca Raton (1995)). Following transplantation into the brain, for example, the transfected cell expresses and secretes an active fragment of prosaposin and, thus, alleviates neuropathic pain. Such a method can be useful to alleviate neuropathic pain as described for the transplantation of cells that secrete substances with analgesic properties (see, for example, Czech and Sagen, Prog. Neurobiol. 46:507-529 (1995)).

The cell can be any cell that can survive when transplanted and that can be modified to express and secrete an active fragment of prosaposin. In practice, the cell should be immunologically compatible with the subject. For example, a particularly useful cell is a cell isolated from the subject to be treated, since such a cell is immunologically compatible with the subject.

A cell derived from a source other than the subject to be treated also can be useful if protected from immune rejection using, for example, microencapsulation or immunosuppression. Useful

microencapsulation membrane materials include alginate-poly-L-lysine alginate and agarose (see, for example, Goosen, Fundamentals of Animal Cell Encapsulation and Immobilization, CRC Press, Boca Raton (1993); Tai and Sun, FASEB J. 7:1061 (1993); Liu et al., Hum. Gene Ther. 4:291 (1993); and Taniguchi et al., Transplant. Proc. 24: 2977 (1992)). For example, pain reduction has been achieved using polymer encapsulated cells transplanted into the rat spinal subarachnoid space (Wang et al., Soc. Neurosci. Abstr. 17:235 (1991)).

For treatment of a human subject, the cell can be a human cell, although a non-human mammalian cell also can be useful. In particular, a human fibroblast, muscle cell, glial cell, neuronal precursor cell or neuron can be transfected with an expression vector to express and secrete an active fragment of prosaposin such as SEQ ID NO: 1. A primary fibroblast can be obtained, for example, from a skin biopsy of the subject to be treated and maintained under standard tissue culture conditions. A primary muscle cell also can be useful for transplantation. Considerations for neural transplantation are described, for example, in Chang, *supra*, 1995.

A cell derived from the central nervous system can be particularly useful for transplantation to the central nervous system since the survival of such a cell is enhanced within its natural environment. A neuronal precursor cell is particularly useful in the method of the invention since a neuronal precursor cell can be

grown in culture, transfected with an expression vector and introduced into an individual, where it is integrated. The isolation of neuronal precursor cells, which are capable of proliferating and differentiating into neurons and glial cells, is described in Renfranz et al., Cell 66:713-729 (1991).

Methods of transfecting cells *ex vivo* are well known in the art (Kriegler, Gene Transfer and Expression: A Laboratory Manual, W.H. Freeman & Co., New York (1990)). For the transfection of a cell that continues to divide such as a fibroblast, muscle cell, glial cell or neuronal precursor cell, a retroviral vector is preferred. For the transfection of an expression vector into a postmitotic cell such as a neuron, a replication-defective herpes simplex virus type 1 (HSV-1) vector is useful (During et al., Soc. Neurosci. Abstr. 17:140 (1991); Sable et al., Soc. Neurosci. Abstr. 17:570 (1991)).

A nucleic acid encoding an active fragment of prosaposin can be expressed under the control of one of a variety of promoters well known in the art, including a constitutive promoter or inducible promoter. See, for example, Chang, *supra*, 1995. A particularly useful constitutive promoter for high level expression is the Moloney murine leukemia virus long-terminal repeat (MLV-LTR), the cytomegalovirus immediate-early (CMV-IE) or the simian virus 40 early region (SV40).

A nucleic acid sequence encoding an active fragment of prosaposin is disclosed herein. For example, a nucleic acid sequence encoding SEQ ID NO: 1 is 5'-TGTGAATTCCTGGTGAAGGAGGTGACCAAGCTGATTGACAACAACAAGACTGAG AAAGAAATACTC-3' (SEQ ID NO: 21)(Dewji et al., Proc. Natl. Acad. Sci. USA 84:8652-8656 (1987)). In order to direct secretion of peptide SEQ ID NO: 1, for example, a nucleic acid encoding a signal sequence, such as the signal sequence of β-lactamase, can be operably linked to SEQ ID NO: 21 as described in Simon et al., J. Cell Biol. 104:1165 (1987).

The invention further provides a method of preventing neuropathic pain in a subject by administering an effective amount of an active fragment of prosaposin to the subject. The method of preventing neuropathic pain is useful when applied prior to a painful event, for example, prior to chemotherapy or surgery that is known to result in neuropathic pain.

The following examples are intended to illustrate but not limit the present invention.

EXAMPLE I

Alleviation of neuropathic pain in Chung model rats

This example describes the effects of bolus intrathecal injection of an active fragment of prosaposin in the Chung experimental model of peripheral neuropathic pain.

Each of the three peptides were obtained in pure form by chemical synthesis, dissolved in sterile PBS and buffered to a neutral pH.

The surgical procedure previously described by Kim and Chung, *supra*, 1992, was performed on male Sprague-Dawley rats weighing 120 to 150 grams to induce an allodynic state. Briefly, the rats were anesthetized with halothane; subsequently, the left L-5 and L-6 spinal nerves were isolated adjacent to the vertebral column and ligated with 6.0 silk suture distal to the dorsal root ganglion. After a ten to fourteen day post operative recovery period, a spinal catheter was introduced. Five days following the second surgery, intrathecal drug administration was accomplished using a gear driven micro-injection syringe connected to a spinal catheter inserted through the foramen magnum. Prior to testing, the rats were placed in clear plastic wire meshed cages and allowed to acclimate.

To assess the 50% mechanical threshold for paw withdrawal, a von Frey hair was applied to the hind foot avoiding the foot pad. Each of the von Frey hairs, which are calibrated to bend at increasing log forces, were pressed perpendicularly to the foot with sufficient force to cause slight bending for a duration of approximately six to eight seconds. A positive response was noted if the foot was sharply withdrawn. Six data points were collected for each point with the maximum and minimum stimulus noted for each time point. The resulting pattern of the responses was tabulated, and the 50%

response threshold was computed. The graph gives the response to the indicated dosage of peptide given as a single intrathecal bolus injection. The X-axis indicates the time after the injection at which point the hypersensitivity to pressure on the foot pad was measured.

All surgically lesioned rats showed tactile allodynia prior to injection with an active fragment of prosaposin. As shown at time zero in Figure 1, the measured threshold was less than 3.0 to 4.0 g in the absence of peptide. Intrathecal injection of 0.7 or 0.07 μg of the prosaposin-derived 22-mer peptide (SEQ ID NO: 1) suppressed allodynia in a dose-dependent fashion. The reduction of allodynia is manifest by the increase in the force threshold as the rats withstand an increasing force before withdrawing the affected foot.

A significant effect was observed by 15 minutes after the injection. The maximum effect was seen 120 minutes post-injection. Rats injected with the highest dose of the prosaposin-derived 22-mer peptide (SEQ ID NO: 1) continued to demonstrate significantly reduced allodynia at the latest time point assayed (180 minutes). Rats that were injected with 0.007 μg prosaposin-derived 22-mer peptide (SEQ ID NO: 1) showed no significant reduction in allodynia. No significant side effects such as sedation were observed at any concentration.

The ability of the prosaposin-derived 14-mer peptide (SEQ ID NO: 2; see Table 1) to relieve allodynia

in Chung model rats also was examined. As shown in Figure 2, the active fragment of prosaposin (SEQ ID NO: 2) was effective in reducing allodynia. The peak effect of the prosaposin-derived 14-mer peptide (SEQ ID NO: 2) was observed 15 to 30 minutes following the injection and returned to the pre-injection value by 60 minutes (Figure 2). No side effects were observed at either concentration of prosaposin-derived 14-mer peptide (SEQ ID NO: 2) tested.

A mutant 22-mer peptide (SEQ ID NO: 8) that differs from the prosaposin-derived 22-mer peptide (SEQ ID NO: 1) by containing an aspartic acid residue instead of an asparagine (see Table 4) also was tested for activity in relieving allodynia in Chung model rats. No change in the allodynic response of the Chung rats was observed following injection of 17.5 μg mutant 22-mer peptide (SEQ ID NO: 8).

Normal rats, which do not experience pain as a result of surgical lesion introduced according to the Chung model, also were injected with an active fragment of prosaposin (SEQ ID NO: 1) and tested for their response to a heat stimulus according to the procedure developed by Bennett and Xie, *supra*, 1988. Briefly, the period of time before the rat withdraws the affected foot from a source of heat is defined as the hot plate latency and is a measure of tolerance to pain caused by a heat stimulus.

An intrathecal catheter was placed into normal male Sprague Dawley rats. Five days after this surgery, rats were injected intrathecally with an active fragment of prosaposin (SEQ ID NO: 1). Rats were examined on the hot plate (52.5°C); hot plate response latencies were measured prior to injection and at various time points up to 180 minutes after the injection. No significant elevation of the hot plate response latency was observed. Thus, the prosaposin-derived peptide SEQ ID NO: 1 does not effect the perception of pain in normal animals.

EXAMPLE II

In vivo uptake of prosaposin-derived peptides by the central nervous system

The results described in this example indicate that prosaposin-derived peptides cross the blood-brain barrier.

An 18-mer peptide (SEQ ID NO: 20) consisting of amino acids 12-29 of saposin C with a tyrosine substituted for valine at position 12 was chemically synthesized on an Applied Biosystems Model 430 peptide synthesizer. The peptide was then radioiodinated by the lactoperoxidase method; 20 x 10^6 cpm radiolabeled peptide were injected into the auricles of rats. The animals were sacrificed after one hour and 24 hours, and the hearts were perfused with isotonic saline in order to remove the blood from the brain.

In order to determine the percentage of peptide uptake, the brain was then counted in a gamma counter. In addition, the brain was homogenized and fractionated into a capillary rich fraction (pellet) and a parenchymal brain fraction (supernatant) after dextran centrifugation (Triguero et al., J. Neurochem., 54:1882-1888 (1990)). This method allows for the discrimination between radiolabeled peptide within blood vessels and that within the brain. After 24 hours, 0.017% of the injected peptide (SEQ ID NO: 20) was detected in whole brain; 75% of the label was in the parenchymal fraction and 25% was in the capillary fraction. At 1 hour, 0.03% of the injected dose was present in whole brain.

The prosaposin-derived peptide SEQ ID NO: 2 also was assayed for ability to cross the blood-brain barrier as follows. A female Sprague-Dawley rat was anesthesized with methoxyflurane, and approximately 20 μg peptide SEQ ID NO: 2 (3.2×10^8 cpm) was injected into the tail vein. After 40 minutes, the rat was sacrificed by ether anesthesia and perfused with about 250 ml PBS through the heart. The total amount of peptide in brain, liver and blood was calculated as a percentage of the injected material as shown in Table 6. In order to determine the localization in brain, the capillary depletion method of Triguero, J. Neurochem. 54:1882 (1990) was used to separate brain tissue into a parenchyma fraction and a brain capillary fraction. The fractionation results showed that 87% of the SEQ ID NO: 2 peptide present in brain was localized to brain parenchyma while 13% was found in brain capillary.

Table 6			
TISSUE	WEIGHT	TOTAL CPM IN TISSUE	PERCENTAGE OF INITIAL CPM
Brain	1.3 gm	161,000	0.050
Liver	8.8 gm	5.2×10^6	1.625
Blood	about 22μl	1.01×10^8	31.6

In a similar experiment in which rats were sacrificed after three hours treatment with SEQ ID NO: 2, 0.06% of the peptide was evident in brain, of which 85% was in the parenchyma. These results demonstrate that at least some of the prosaposin-derived peptide SEQ ID NO: 2 crossed the blood brain barrier and was concentrated in the brain parenchyma rather than the vascular endothelium (blood vessels). The percentage of peptide which crossed the blood brain barrier is in the midrange of peptides which cross the barrier as set forth in Banks, *supra*, 1992.

In order to determine the percentage of intact material in the brain, liver and blood, radiolabelled material (SEQ ID NO: 2) isolated from the tissues was analyzed by high pressure liquid chromatography. To normalize for degradation during processing of tissue homogenates, peptide SEQ ID NO: 2 was added to tissue homogenates. The extent of degradation observed with the added peptide material was used to normalize for degradation during tissue processing. After normalization, the results were as follows: SEQ ID NO: 2 was about 60% intact in brain; about 80% intact in liver

and about 40% intact in blood. In a second experiment, peptide SEQ ID NO: 2 was about 68% intact in brain. These results indicate that the peptide SEQ ID NO: 2 crosses the blood brain barrier and is largely intact in brain.

EXAMPLE III

Alleviation of neuropathic pain in diabetic rats

This example describes the effects of intraperitoneal administration of a peptide having the sequence of SEQ ID NO: 2 in a rat model of diabetic neuropathy.

Rats were made diabetic by a single intraperitoneal injection of streptozotocin (50mg/kg body weight, freshly dissolved in 0.9% sterile saline) to ablate pancreatic β cells and induce insulin deficiency as described in Calcutt et al., Pain 68:293-299 (1996). Two days later, diabetes was confirmed in streptozotocin-injected rats by measuring blood glucose levels. Streptozotocin-injected animals with a blood glucose concentration below 15 mmol/l were excluded from subsequent studies, according to the commonly accepted definition of non-fasting hyperglycemia in studies of diabetes in rats.

Both diabetic and control rats were studied at 8 weeks by analyzing the behavioral response to the noxious chemical formalin as an indicator of allodynia (Calcutt et al. *supra*, 1996). Briefly, rats received a

subcutaneous injection of freshly-prepared formalin (50 µl of 0.5% solution in sterile saline) into the dorsal surface of the right hind paw. This concentration of formalin induces sub-maximal behavioral responses in control rats and allows detection of hyperalgesia in diabetic rats during phases Q and 2 (Calcutt et al., Eur. J. Pharmacol. 285:189-197 (1995). Animals were transferred to an observation chamber constructed to allow continuous visualization of the paws. The number of flinches during one minute periods were counted at 5 minute intervals for the next 60 minutes by an observer who was unaware of the treatment group of each animal. Phase 1 was defined as the initial measurement of flinching (1-2 and 5-6 minutes post injection); the Q (quiescent) phase as the measurements made at 10-11, 15-16 and 20-21 minutes; and Phase 2 as all subsequent measurements post injection, as previously defined for studies of diabetic rats (see, for example, Malmberg et al., Neurosci. Lett. 161:45-48 (1993)). Comparisons of activity during each phase were made by summing the flinches at measurement points within the phase. Diabetic rats gave an abnormal flinch response, as has been reported previously.

Peptide SEQ ID NO: 2 was obtained in pure form by chemical synthesis, dissolved in sterile PBS and buffered to a neutral pH. Diabetic rats were divided in two groups of four animals each, which were administered saline or peptide SEQ ID NO: 2, respectively. Two hours before treatment with 0.5% formalin, the diabetic rats were treated with saline or 200 µg/kg peptide SEQ ID NO:

2 using intraperitoneal administration. As shown in Figure 3, administration of SEQ ID NO: 2 completely prevented the abnormal flinch response in Phase 1 and ameliorated the response in Phase 2 by 70%. Thus, parenteral administration of peptide SEQ ID NO: 2 alleviated the pain from formalin injection in a rat model of painful diabetic neuropathy.

EXAMPLE IV

Stimulation of neurite outgrowth *in vitro*

This example describes the use of a peptide having the sequence of SEQ ID NO: 2 in stimulating neurite outgrowth *in vitro*.

NS20Y neuroblastoma cells are grown in DMEM containing 10% fetal calf serum (FCS). Cells are removed with trypsin and plated in 30 mm petri dishes onto glass coverslips. After 20 to 24 hours, the medium is replaced with 2 ml DMEM containing 0.5% fetal calf serum with 0, 0.5, 1, 2, 4 or 8 ng/ml of a peptide having sequence SEQ ID NO: 2 or a scrambled control peptide. Cells are cultured for an additional 24 hours, washed with PBS and fixed with Bouin's solution (saturated aqueous picric acid/formalin/acetic acid 15:5:1) for 30 minutes. After fixative is removed with PBS, neurite outgrowth is scored under a phase contrast microscope. Cells exhibiting one or more clearly defined neurites equal to or longer than one cell diameter are scored as positive for neurite outgrowth. At least 200 cells are scored in different positions of each dish to determine the percentage of

neurite bearing cells with each peptide assayed in duplicate.

The peptide shown in SEQ ID NO: 2 significantly increases neurite outgrowth in NS20Y cells as compared to a scrambled control peptide having the same amino acids in a different order. Increased neurite outgrowth is evident using as little as 0.5 ng/ml peptide.

EXAMPLE V

Inhibition of neural cell death *in vitro*

This example describes the use of a peptide having the sequence of SEQ ID NO: 2 in inhibiting neural cell death *in vitro*.

NS20Y cells are plated as described in Example IV and grown on glass coverslips in 0.5% fetal bovine serum for 2 days in the presence or absence of 8 ng/ml of a peptide having the sequence shown as SEQ ID NO: 2 or a scrambled control peptide. Media is removed and 0.2% trypan blue in PBS is added to each well. Dead cells stain blue with the trypan blue dye and are scored as a percentage of the total on an inverted microscope, counting 400 cells in four areas of each well. The average error of duplicates is ±5%. The peptide shown as SEQ ID NO: 2 substantially reduces the number of trypan blue-positive (dead) cells. This indicates that a peptide having the sequence SEQ ID NO: 2 can inhibit programmed cell death.

EXAMPLE VI

Ex vivo myelination assay

This example describes the use of a peptide having the sequence of SEQ ID NO: 2 in stimulating neurite outgrowth *ex vivo* and in promoting myelination.

Newborn mouse cerebellar explants are prepared according to Satomi, Zool. Sci. 9:127-137 (1992). Neurite outgrowth and myelination are observed over 22 days in culture, during the period when the newborn mouse cerebellum normally undergoes neuronal differentiation and myelination begins. On the second day after preparation of the explants, the peptide having the sequence of SEQ ID NO: 2 is added to three explants at a concentration of 10 μg/ml and a scrambled control peptide is added to three explants at a concentration of 10 μg/ml. Neurite outgrowth and myelination in three control and three treated explants is assessed under a bright field microscope with a video camera. On the eighth day, cultures containing the peptides are thinner and more spread out than control cultures. On day 15, cultures treated with peptide SEQ ID NO: 2 contain many cells with long projections at the periphery of the explant. Such projections are absent or less prominent in control cultures. Cultures treated with peptide SEQ ID NO: 2 contain significantly more myelinated axons in the subcortical white matter at 22 days compared to control explants. Thus, the peptide of the invention induces myelination in differentiating cerebellum *ex vivo*.

EXAMPLE VII

Inhibition of demyelination

Reduction of Schwann cell death is correlated with inhibition of demyelination. Schwann cells contain an extensive myelin sheath. The addition of the peptide shown in SEQ ID NO: 2 to Schwann cells in culture reduces Schwann cell death in a dose-dependent manner not seen with a control scrambled peptide. Thus, a peptide of the invention having the sequence of SEQ ID NO: 2 can inhibit demyelination.

EXAMPLE VIII

Treatment of traumatic ischemic CNS lesions

Humans with traumatic lesions to the spinal cord receive an intracerebrospinal injection or direct injection of about 100 μg/ml of the peptide shown in SEQ ID NO: 2 in a sterile saline solution or in depot form to enable slow, continuous release of the peptide at the lesion site. Improvement is assessed by gain of motor nerve function such as increased limb movement. Treatments are repeated until no further improvement occurs.

EXAMPLE IX

Treatment of demyelination disorders

Patients diagnosed with early stage MS are given a peptide having the sequence shown in SEQ ID NO: 2 by direct intravenous injection into the cerebrospinal

fluid using the same dose range as in Example VIII. Dosages are repeated daily or weekly and improvement in muscle strength, musculoskeletal coordination and myelination (as determined by MRI) is observed.

EXAMPLE X

Treatment of sensory neuropathy

Mice were administered taxol in order to induce sensory neuropathy. The taxol-treated mice were administered 100 μg/kg, 200 μg/kg or 1 mg/kg of the prosaposin-derived peptide SEQ ID NO: 2. The loss of thermal sensation was measured using a Hargreaves sensory testing apparatus as an indicator of sensory neuropathy. Each of the three doses of peptide SEQ ID NO: 2 administered were effective in inhibiting loss of thermal sensation in taxol-treated mice. The prosaposin-derived 22-mer peptide SEQ ID NO: 1 also was similarly assayed and found to be effective in inhibiting less of thermal sensation in the taxol-treated mice. These results show that prosaposin-derived peptides such as SEQ ID NO: 1 and SEQ ID NO: 2 can be used to effectively inhibit sensory neuropathy.

Although the invention has been described with reference to the examples above, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: The Regents of the University of California

(ii) TITLE OF INVENTION: Methods of Alleviating Neuropathic Pain Using Prosaposin-Derived Peptides

(iii) NUMBER OF SEQUENCES: 21

(iv) CORRESPONDENCE ADDRESS:
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(B) STREET: 4370 La Jolla Village Drive, Suite 700
(C) CITY: San Diego
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 92122

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE: 05-MAR-1997
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Campbell, Cathryn A.
(B) REGISTRATION NUMBER: 31,815
(C) REFERENCE/DOCKET NUMBER: FP-UD 2474

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (619) 535-9001
(B) TELEFAX: (619) 535-8949

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Cys Glu Phe Leu Val Lys Glu Val Thr Lys Leu Ile Asp Asn Asn Lys
1 5 10 15

Thr Glu Lys Glu Ile Leu
20

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 2
(D) OTHER INFORMATION: /note= "Xaa is D-alanine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Thr Xaa Leu Ile Asp Asn Asn Ala Thr Glu Glu Ile Leu Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Leu Ile Asp Asn Asn Lys Thr Glu Lys Glu Ile Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Cys Gln Phe Val Met Asn Lys Phe Ser Glu Leu Ile Val Asn Asn Ala
1 5 10 15

Thr Glu Glu Leu Leu Tyr
20

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Cys Gln Leu Val Asn Arg Lys Leu Ser Glu Leu Ile Ile Asn Asn Ala
1 5 10 15

Thr Glu Glu Leu Leu
20

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Cys Glu Tyr Val Val Lys Lys Val Met Leu Leu Ile Asp Asn Asn Arg
1 5 10 15

Thr Glu Glu Lys Ile Ile
20

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Cys Glu Phe Val Val Lys Glu Val Ala Lys Leu Ile Asp Asn Asn Arg
1 5 10 15

Thr Glu Glu Glu Ile Leu
20

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Cys Glu Phe Leu Val Lys Glu Val Thr Lys Leu Ile Asp Asp Asn Lys
1 5 10 15

Thr Glu Lys Glu Ile Leu
20

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Thr Lys Leu Ile Asp Asn Asp Lys Thr Glu Lys Glu Ile Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Thr Lys Ser Ile Asp Asn Asn Lys Thr Glu Lys Glu Ile Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Tyr Val Lys His Gln Gly Leu Asn Lys Asn Ile Asn Leu Asp Ser Val
1 5 10 15

Asp Gly Val Pro
20

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Glu Ala Leu Ala Glu Asn Asn Leu Asn Leu Pro Lys Met Ala Gly
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys Asn Pro Lys Leu
1 5 10 15

Thr

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Phe Tyr Leu Arg Asn Asn Gln Leu Val Ala Gly Thr Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Ala Glu His Cys Ser Leu Asn Glu Asn Ile Thr Val Pro Asp Thr Lys
1 5 10 15

Val

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Tyr Thr Ala Gln Gly Glu Pro Phe Pro Asn Asn Val Glu Lys Leu Cys
1 5 10 15

Ala Pro

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Phe Asn Lys Ile Glu Ile Asn Asn Lys Leu Glu Phe Glu Ser Ala
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Arg Pro Asn Ile Leu Gly Leu Arg Asn Asn Ile Tyr Cys Met Ala Gln
1 5 10 15

Leu Leu

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Tyr Lys Glu Val Thr Lys Leu Ile Asp Asn Asn Lys Thr Glu Lys Glu
1 5 10 15

Ile Leu

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TGTGAATTCC TGGTGAAGGA GGTGACCAAG CTGATTGACA ACAACAAGAC TGAGAAAGAA 60

ATACTC 66

We claim:

1. A prosaposin-derived peptide having from about 14 to about 50 amino acids and including the sequence shown in SEQ ID NO: 2.

2. The peptide of Claim 1, wherein said peptide has the sequence shown in SEQ ID NO: 2.

3. A pharmaceutical composition for therapy of neural or demyelination disorders in neural tissue comprising the peptide of Claim 1 or Claim 2 in a pharmaceutically acceptable carrier.

4. The composition of Claim 3 in a controlled release formulation.

5. The composition of Claim 3 in a liposomal form.

6. The composition of Claim 3 in a lyophilized form.

7. The composition of Claim 3 in a unit dosage form.

8. A method of alleviating neuropathic pain in a subject, comprising administering an effective amount of an active fragment of prosaposin to the subject.

9. The method of claim 8, wherein said active fragment has an amino acid sequence selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2.

10. The method of claim 8, wherein said neuropathic pain results from a peripheral nerve disorder.

11. The method of claim 10, wherein said peripheral nerve disorder is selected from the group consisting of neuroma; nerve compression; nerve crush, nerve stretch and incomplete nerve transsection; mononeuropathy and polyneuropathy.

12. The method of claim 8, wherein said neuropathic pain results from a disorder selected from the group consisting of a disorder of dorsal root ganglia, spinal cord, brainstem, thalamus and cortex.

13. The method of claim 8, wherein said administering is selected from the group consisting of: intravenous, intramuscular, intradermal, subcutaneous, intracranial, intracerebrospinal, topical, oral, transdermal, transmucosal and transnasal.

14. A method of preventing neuropathic pain in a subject, comprising administering an effective amount of an active fragment of prosaposin to the subject.

15. The method of claim 14, wherein said active fragment has an amino acid sequence selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2.

16. A method of alleviating neuropathic pain in a subject, comprising administering to said subject an effective amount of a peptide having up to about 50 amino acids and including the active neurotrophic region contained within one of the following sequences: SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18 or SEQ ID NO: 19.

17. Use of a peptide having up to about 50 amino acids and including the active neurotrophic region contained within one of the following sequences: SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18 and SEQ ID NO: 19 for treatment of neuropathic pain.

18. A method of stimulating neurite outgrowth, inhibiting neural cell death, promoting myelination or inhibiting demyelination, comprising the step of contacting neuronal cells with a composition comprising

an effective stimulating or inhibiting amount of a peptide having between about 14 and about 50 amino acids and including the sequence shown in SEQ ID NO: 2.

19. The method of Claim 18, wherein said composition comprises a peptide having the sequence shown in SEQ ID NO: 2.

20. The method of Claim 18, wherein said neuronal cells are contacted *in vitro*.

21. The method of Claim 18, wherein said neuronal cells are contacted *in vivo*.

22. Use of the peptide of Claim 1 or Claim 2 for treatment of neural or demyelination disorders of neural tissue.

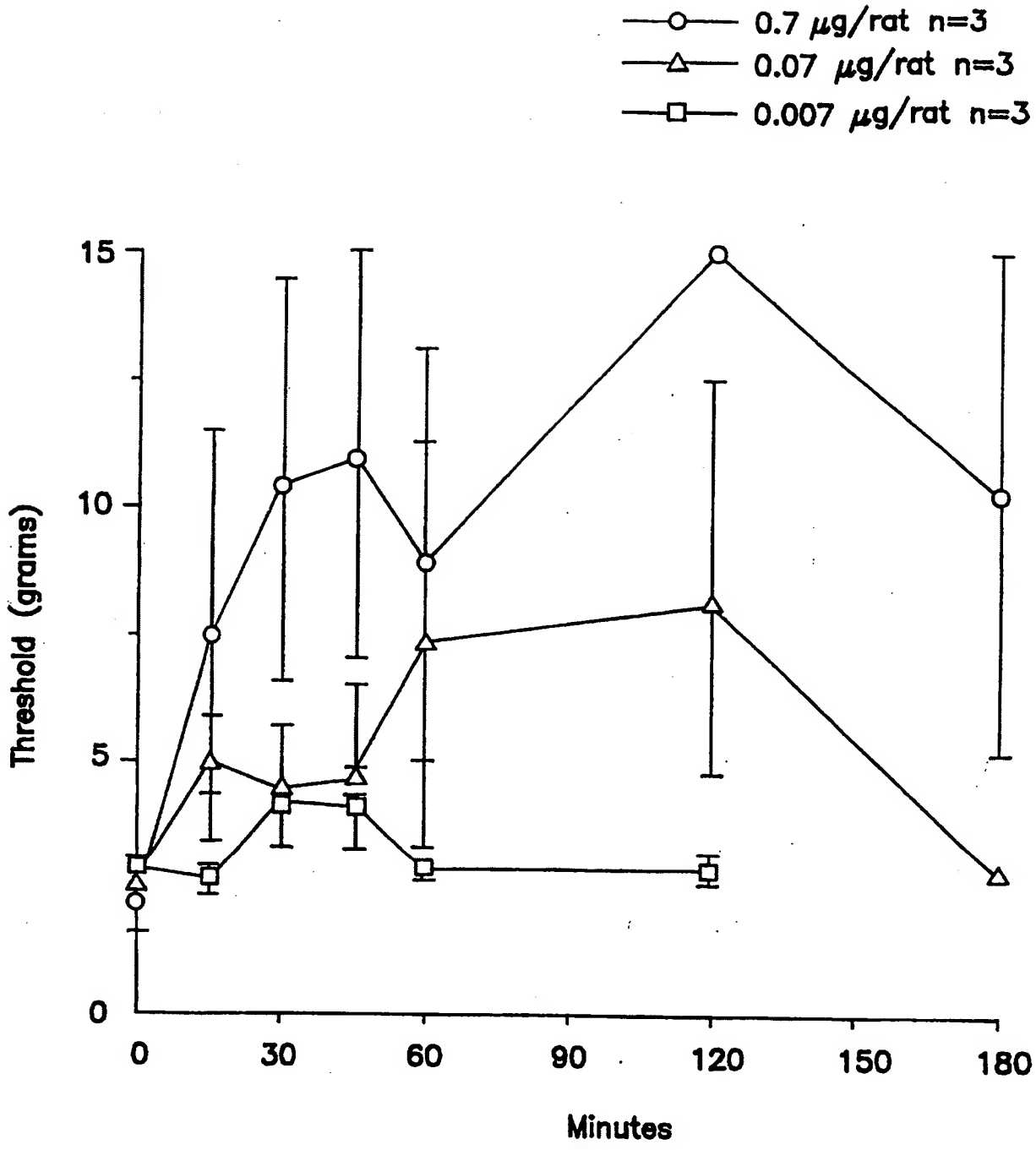
23. A method of inhibiting sensory or motor neuropathy, comprising the step of contacting neuronal cells with a composition comprising an effective inhibiting amount of an active fragment of prosaposin.

24. The method of claim 23, wherein said active fragment of prosaposin is a peptide having between about 14 and about 50 amino acids and including the sequence shown in SEQ ID NO: 2.

25. The method of Claim 24, wherein said active fragment of prosaposin is a peptide having the sequence shown in SEQ ID NO: 2.

26. The method of Claim 23, wherein said neuronal cells are contacted *in vitro*.

27. The method of Claim 23, wherein said neuronal cells are contacted *in vivo*.


0.7 µg/rat n=3
0.07 µg/rat n=3
0.007 µg/rat n=3
Threshold (grams)
15
10
5
0
0
30
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90
120
150
180
Minutes

FIG. 1

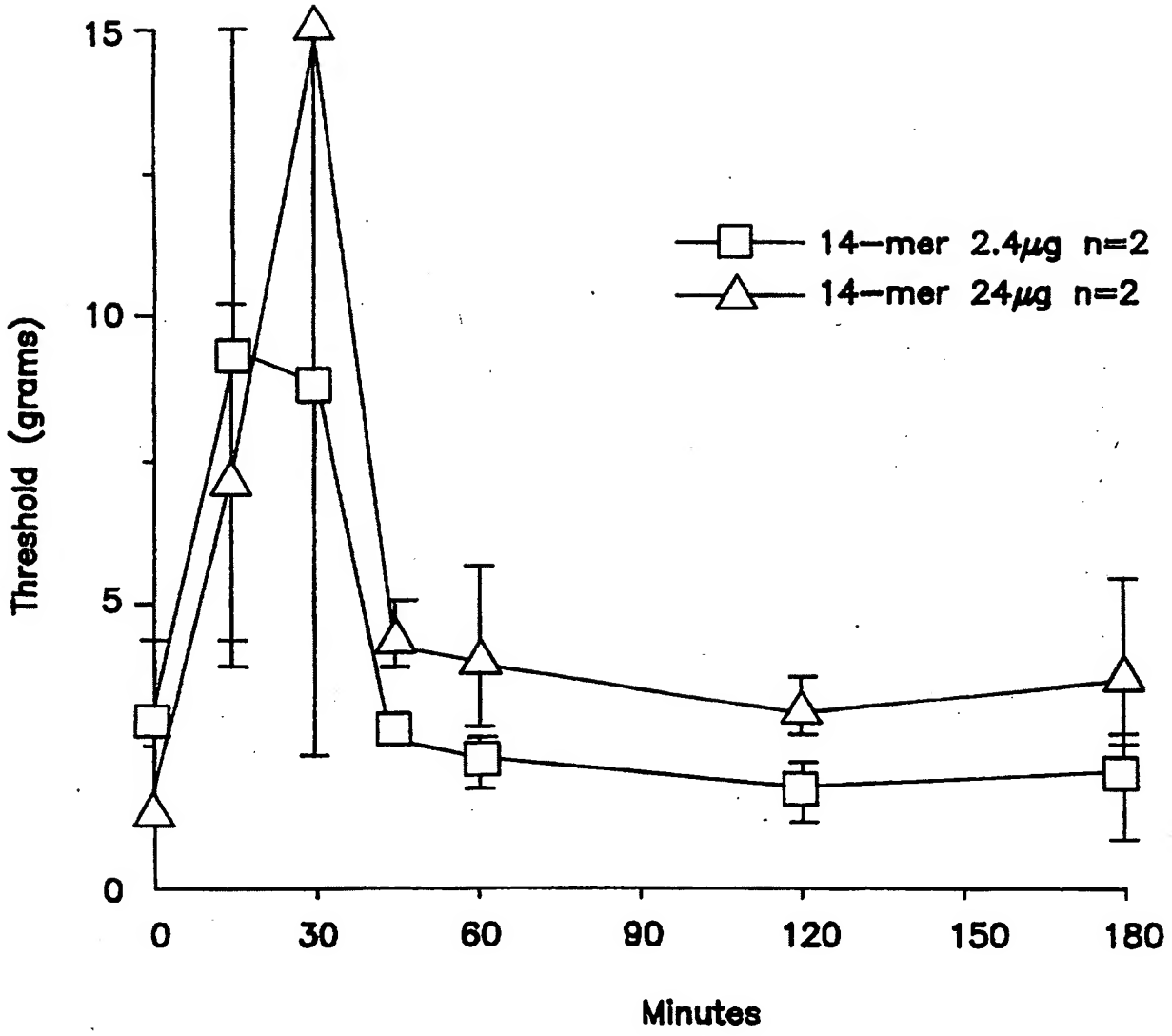
15
10
5
0
Threshold (grams)
0
30
60
90
120
150
180
Minutes
14–mer 2.4μg n=2
14–mer 24μg n=2

FIG. 2

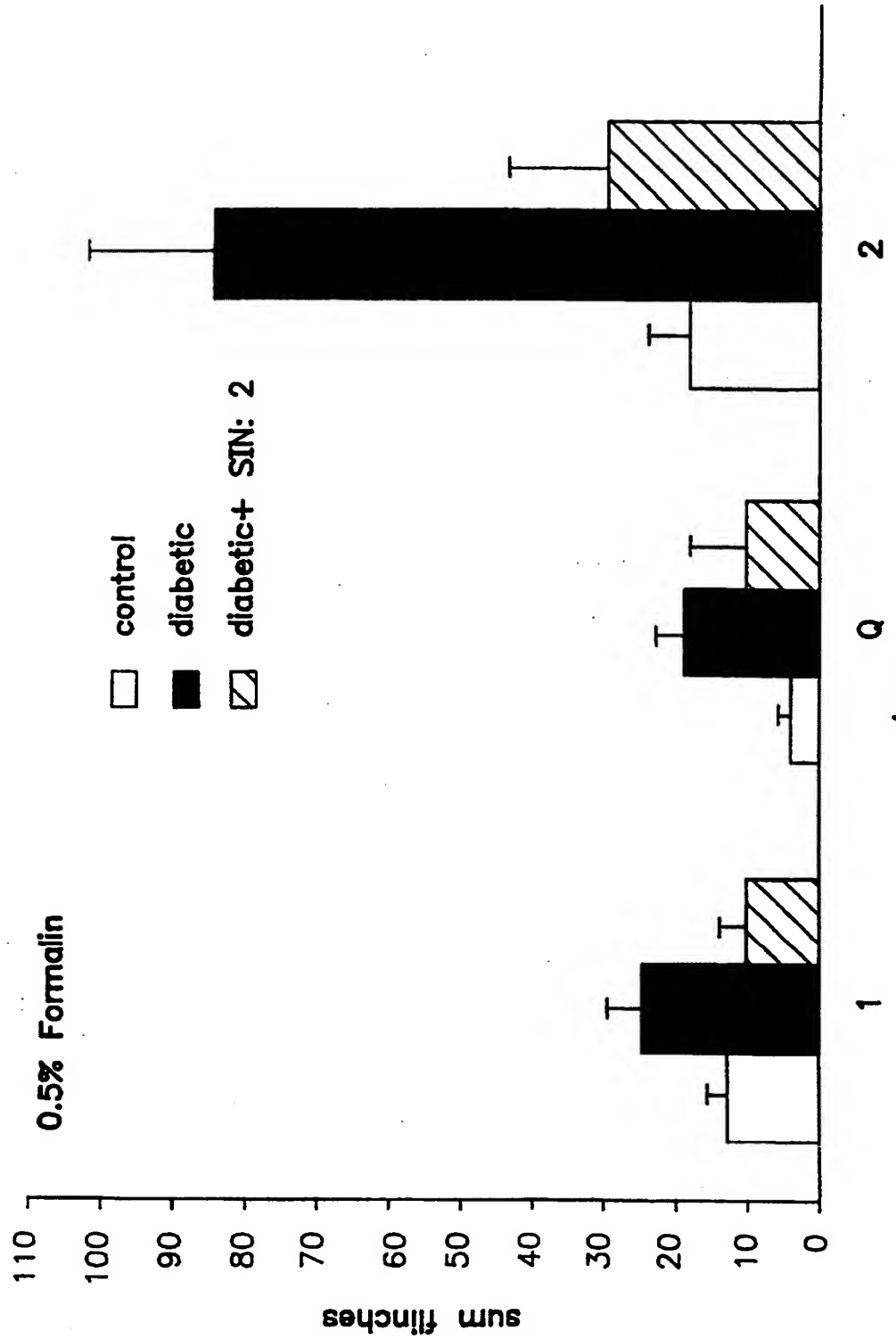

FIG. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/04143

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :C07K 5/00; A61K 38/10, 38/17
US CL :530/324, 327; 514/12, 14
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 530/324, 327; 514/12, 14

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
BIOSIS, MEDLINE, CAPLUS, APS, IntelliGenetics
search terms: prosaposin, neur?, pain

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	O'BRIEN et al. Identification of the Neurotrophic Factor Sequence of Prosaposin. The FASEB Journal. May 1995, Vol. 9, pages 681-685, especially page 682.	1-3, 18-20 --- 4-7
Y	US 5,470,582 A (SUPERSAXO et al) 28 November 1995, columns 2-10.	4, 6-7
X, P	US 5,571,787 A (O'BRIEN et al) 05 November 1996, columns 1-22.	1-7, 10-13, 18-27
Y	SANO et al. Protection by Prosaposin Against Ischemia-Induced Learning Disability and Neuronal Loss. Biochemical and Biophysical Research Communications. 28 October 1994, Vol. 204, No. 2, pages 994-1000, see entire document.	13, 18-27

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 JUNE 1997	28 JUL 1997

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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